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**IN-DEPTH REVIEW** 

# MOLECULAR BIOLOGY IN YOUNG WOMEN WITH BREAST CANCER: FROM TUMOR GENE EXPRESSION TO DNA MUTATIONS

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#### ABSTRACT

Young women with breast cancer (YWBC) represent roughly 15% of breast cancer (BC) cases in Latin America and other developing regions. Breast tumors occurring at an early age are more aggressive and have an overall worse prognosis compared to breast tumors in postmenopausal women. The expression of relevant proliferation biomarkers such as endocrine receptors and human epidermal growth factor receptor 2 appears to be unique in YWBC. Moreover, histopathological, molecular, genetic, and genomic studies have shown that YWBC exhibit a higher frequency of aggressive subtypes, differential tumor gene expression, increased genetic susceptibility, and specific genomic signatures, compared to older women with BC. This article reviews the current knowledge on tumor biology and genomic signatures in YWBC.

Key words: Breast cancer. Young women. Molecular biology. Hereditary breast cancer.

#### INTRODUCTION

Breast cancer (BC) is the most common malignancy in women worldwide, representing about 25% of the cancer cases, and is the leading cause of cancer-related deaths in most countries<sup>1</sup>. In developed regions, such as the European Union and North America, the occurrence of malignant breast tumors predominates during the fifth decade of life<sup>1</sup>. However, 5-8% of cases occur in women younger than 45 years of age<sup>2</sup>. In contrast, the reported incidence rates of young women with BC (YWBC) in developing countries are as high as 10-15% and also show a higher mortality rate<sup>2-5</sup>. The age definition of YWBC varies in the literature, referring to women under the ages of 35, 40, or 45 as "young"<sup>6</sup>. However, there seems to be a biological and clinically meaningful difference among premenopausal women with BC onset by age < 40 years, suggesting this age as a relevant cut-point<sup>6</sup>. Further, YWBC represents a challenge for public health due to its high mortality rate, loss of potential quality-adjusted life years, delayed diagnosis, treatment complexity, and costs. These reasons have opened the field of new basic and clinical research on the tumor biology of YWBC<sup>7-10</sup>. This review focuses on the current knowledge of the histopathological features, the genomic signatures, and molecular biology of the tumors in YWBC.

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#### HISTOPATHOLOGIC FEATURES OF TUMORS IN YOUNG WOMEN

Young age is considered an independent negative prognostic factor for local recurrence<sup>11-13</sup>, contralateral BC<sup>14,15</sup>, and overall outcomes<sup>16,17</sup>. In addition, YWBC patients report a higher incidence of a family history of cancer, particularly BC<sup>18,19</sup>, suggesting a genetic component involved in the etiology of early-onset BC<sup>19,20</sup>.

Breast tumors in young women have been described as being more aggressive than in their older counterparts<sup>6,21</sup>. This aggressiveness may be in part explained by the more frequent finding of a higher histological tumor grade and proliferation index. Tumors from young women express higher levels of the cellular proliferation associated antigen Ki67<sup>22,23</sup>. Furthermore, the other well-known prognostic biomarkers, including estrogen receptor (ER) and progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2), differ in young women compared to older women<sup>6,10,23-26</sup>.

In the Prospective Study of Outcomes in Sporadic and Hereditary Breast Cancer study, Copson et al. described the histopathological features of a cohort of 2956 patients < 40 years with BC. They reported a median age at diagnosis of 36 years, median tumor size of 22 mm, and up to 50% of patients had axillary lymph node metastases at diagnosis. Moreover, 59% of the tumors were high grade, 34% ER-negative (ER–), and 24% HER2-positive (HER2+). Patients with ER– tumors were associated with a worse 5-year overall survival (OS) compared with ER-positive (ER+) tumors<sup>27</sup>.

Regarding the molecular phenotype according to ER, PR, HER2 and Ki67 expression, Collins et al.<sup>26</sup> analyzed the different subtypes of BC among 399 women aged ≤40 years. They found a 35% rate of luminal B tumors, defined as ER+, PR+, and HER2+ or both ER+ and PR+, HER2– and Grade 3, whereas the rate of luminal A tumors, defined as ER+ and PR+, HER2– and Grade 1 or 2, was only 33%. HER2 enriched tumors accounted for 11% of the patients, and triple negative phenotype was found in 21% of the cohort. In Hispanic population, Villarreal-Garza et al.<sup>16</sup> found a similar distribution of BC subtypes and confirmed the worst 5-year OS for young women with luminal B subtype compared to older patients (79% vs. 85%)<sup>16</sup>. Fig. 1 describes the BC subtypes according to age, determined by gene expression profiling.

# MOLECULAR FEATURES OF TUMORS IN YOUNG WOMEN

#### Tumor gene expression profiling

During the last decade, significant efforts to describe the molecular biology of early-onset BC have been made. Anders et al.<sup>20,28</sup> evaluated somatic gene expression profiling in breast tumor tissue in a cohort of 200 young women ( $\leq$ 45 years) and an older age cohort of 211 patients ( $\geq$ 65 years). Using genomic mRNA expression analysis, they showed that tumors in young women had significantly lower mRNA expression of ER $\alpha$ , ER $\beta$ , and PR, and higher HER2 and epidermal growth factor receptor (EGFR) expression. Further, in multivariate analysis of the young women's cohort, lower mRNA expression of ER and higher expression of EGFR predicted inferior disease-free survival. Moreover, exploratory gene set enrichment analysis revealed 367 genes differentially expressed between tumors from young women and their older counterparts. These included genes involved in immune regulation, mammalian target of rapamycin/rapamycin signaling, hypoxia-regulating genes, BRCA1, stem cells, apoptosis, histone deacetylase, and growth and differentiation pathways such as Myc, E2F, Ras,  $\beta$ -catenin, AKT, P53,

> 65 years
53-64 years
41-52 years
40 years
18%
17%
17%
28%
31%
27%
27%
27%
29%
37%
Luminal-A
Luminal-B
HER2
Basal

Figure 1. Breast cancer subtypes according to age, determined by gene expression profiling.

phosphatase and tensin homolog (PTEN), and mitogen-activated protein kinase (MAPK), with potential prognostic and therapeutic significance in YWBC<sup>20</sup>. These results showed, for the first time, that breast tumors in young women share a particular gene expression pattern which may influence its characteristic biologic behavior<sup>28</sup>.

Azim et al.<sup>29</sup> conducted a pooled gene expression analysis on two datasets including 1188 and 2334 patients with nearly 50 genes that were related to early-onset BC. The analysis was adjusted for differences in BC molecular subtype, histological grade, tumor size, and nodal status. Results on the first dataset ( $\leq$ 40 years, n = 191) showed that independent of subtype, grade and stage, younger patients have a higher expression of RANK-ligand and c-kit, in addition to mammary stem cell luminal progenitors and BRCA1 mutation signatures. Furthermore, there was more disruption of MAPK and phosphoinositide 3-kinase (PI3K) pathways and lower expression of BRCA1 and several apoptosis-related genes, particularly FAS. The same findings were reproduced in an independent dataset that included 260 patients who were aged ≤40 years<sup>30</sup>.

Colak et al.<sup>31</sup> analyzed the expression signatures of breast tumors in Middle-Eastern YWBC using genomewide gene expression assays. They compared the transcriptome from BC tumors in three different age cohorts from young women (< 45 years), pre-elderly women (45-55 years) and elderly patients (> 55 years). They identified 63 genes with distinct expression patterns in young women, including those associated with PI3K/ Akt, MYC, nuclear factor kappa B, transforming growth factor-alpha, ErbB2, and interleukin (IL1)/IL1R signaling pathways, which may promote angiogenesis, tumor growth, and metastasis, leading to the aggressive phenotype observed in young women.

# Somatic gene expression profiling during pregnancy and lactation

Pregnancy-associated BC is a particular issue in YWBC. The female breast is a tissue that changes during different biological stages; in young women, the constant fluctuations of steroid hormones over a lifetime affect mammary tissue, which contains a high amount of enriched immature mammary cell populations (stem cells and progenitors), that increase during pregnancy and breastfeeding<sup>32</sup>. Collectively, these events might raise the risk of BC transformation through genome instability, increasing the probability of random genetic mutations and reducing immune surveillance<sup>32</sup>. Reproductive history impacts the prognosis of YWBC; in this regard, patients diagnosed within the next 5 years postpartum have a worse prognosis than nulliparous women or than those diagnosed during pregnancy<sup>33</sup>.

Azim et al.<sup>34</sup> compared two groups of YWBC, pregnant and nonpregnant. Among the pregnant group, tumors had a higher expression of *PD1*, *PD-L1*, and 54 genes related to *SRC*, insulin-like growth factor, and  $\beta$ -catenin. The expression of these genes appeared to increase during gestation in the normal breast tissue, emphasizing on the potential effects of the breast microenvironment on tumor phenotype<sup>34</sup>.

There is a temporary increase in BC risk within the following 5 years postpartum due to mammary gland involution, which is considered a risk factor for tumorigenesis and tumor progression<sup>35,36</sup>. In a murine model, Lyons et al.<sup>33</sup> found that mammary gland involution is a driving force for tumor progression. In this model, human BC cells were injected to involuting mouse mammary glands; the authors observed the progression from ductal *in situ* carcinoma to invasive ductal carcinoma, forming large tumors with abundant fibrillar collagen content, high cyclooxygenase-2 expression (*COX-2*), and an invasive tumor microenvironment. Furthermore, they found that pharmacological inhibition of COX-2 reduced the collagen fibrinogenesis, tumor growth, and cell infiltration to the lung<sup>33</sup>.

These experimental studies suggest that changes occurring during the peripartum period may impact on the biology of BC development in young women. These changes are probably induced by hormonal and inflammatory factors and the resulting adjustment of the breast microenvironment. However, data are still insufficient to conclude that such effects play a fundamental role in carcinogenesis and tumor biology<sup>34</sup>.

# Micro-RNA (mi-RNA) expression profiles in YWBC

miRNAs are short non-coding RNA sequences that regulate gene expression by complementary binding to target mRNA transcripts, usually resulting in transcriptional repression or target degradation<sup>37</sup>. Functional studies have confirmed that miRNA dysregulation is involved in the initiation, progression, and metastasis of human cancers, including BC, making miRNAs a potential therapeutic target<sup>38,39</sup>. Some experimental data suggest that there is a particular miRNA expression pattern in YWBC<sup>40-42</sup>.

Peña-Chilet et al.<sup>40</sup> studied the miRNA profile of 45 YWBC (defined as  $\leq$ 35 years) compared to older women and found a unique expression of 96 miRNAs according to age (p < 0.05). The research group validated the expression of 6 miRNAs, finding upregulation of miRNA-1228\*, miRNA-3196, miRNA-1275, and miRNA-1207, and downregulation of miRNA-139-5p and miRNA-92b. These miRNAs are involved in pathways related to cell motility and apoptosis, mitotic and proliferation regulatory mechanisms, and the PI3K and IGFR signaling, all these features associated with higher metastatic capacity<sup>40</sup>.

Li et al.<sup>41</sup> analyzed the expression of miRNA-146a and miRNA-146b in 120 YWBC and 130 patients with breast fibroadenomas. The levels of miRNAs were lower in BC compared to fibroadenomas and precancerous breast tissue (p < 0.005). In breast tumor tissue, the downregulation of miRNA-14a/b was associated with ER/PR-, HER2-, Ki-67 index  $\ge 20\%$ , tumor size > 2 cm, distant metastasis, lymph node metastasis, advanced clinical tumor, node and metastasis stages (III-IV), and basal-like phenotype<sup>41</sup>. In BC, these miRNAs are associated with down-regulation of *BRCA1* through binding to the 3'UTR of this gene<sup>43</sup>. These results suggest that miRNA-146a/b could be a potential biomarker for YWBC.

Nassar et al.<sup>42</sup> studied the miRNA expression in 57 breast tumor samples from young women. They found a significant upregulation of miRNA-155, miRNA-21, and miRNA-148b along with downregulation of miR-NA-10b, which positively correlated with ER and PR expression. Moreover, miRNA-155 was overexpressed in women diagnosed after the age of 40, suggesting that it could be a potential biomarker for age at diagnosis42. The expression of miRNA-21 has been reported to contribute to invasion and metastasis by targeting tumor suppressors PTEN, PDCD4, mammary serine protease inhibitor (Maspin), and a number of other genes involved in tumor proliferation and metastasis<sup>44,45</sup>. The expression of miRNA-148b has been associated with BC progression in a relapse-associated miRNA signature by targeting ITGA5, ROCK1, PIK3CA, NRAS, and CSF1<sup>46</sup>. The miRNA-10b inhibits translation of mRNA encoding homeobox D10, which leads to increased expression of the pro-metastatic gene  $RHOC^{47}$ .

Recently, miRNAs have risen as potential biomarkers and key molecular regulators of the pathogenesis and progression of BC. miRNAs can be detected in blood, increasing their potential as noninvasive biomarkers. Furthermore, there is a growing body of evidence of miR-NAs' association with drug resistance, again suggesting a role as therapeutic targets<sup>48</sup>. Although research has clarified the role of miRNAs in BC, it is important to standardize the experimental protocols to validate these results before translation to the clinical arena<sup>49</sup>.

# Epigenetic profile in YWBC

The differentiation and diversity of cellular functions are regulated by many factors, including the methylation-regulated pattern of gene expression. Aberrant methylation patterns in tumor cells are associated with the development of BC<sup>50,51</sup>. Wong et al.<sup>52</sup> demonstrated that the constitutional DNA methylation of the *BRCA1* promoter was significantly associated with an increased risk of early-onset BC in women under the age of 40 years. The clinical features of women with constitutionally silenced *BRCA1* were similar to the characteristics of patients with germline mutated *BRCA1*, indicating that methylation patterns can mimic germline mutations<sup>52</sup>.

Scott et al.51 examined the role of methylation in a broad set of high and moderate BC susceptibility genes in 43 women diagnosed with BC before the age of 40 years negative for germline mutations in the genes included in the study. The methylation patterns across the promoter regions of BRCA1, BRCA2, Ataxia Telangiectasia Mutated (ATM), partner and localizer of BRCA2 (PALB2), CDH1, TP53, FANCM, checkpoint serine threonine kinase 2 (CHEK2), MLH1, MSH2, MSH6, and PMS2 were analyzed in blood and tumor DNA samples. There were significant differences between blood and tumor DNA methylation patterns. In tumors, there was an increased methylation of BRCA1, BRCA2, ATM, CHEK2, MLH1, MSH2, and MSH6, while methylation in CDH1 was increased in the blood. PMS2 was hypomethylated, and PALB2 was hypermethylated in most of the tumor samples<sup>40,43,53</sup>. This study yielded interesting insights on specific methylation patterns in the main genes involved in BC carcinogenesis. Further

research is necessary to confirm these findings in larger groups of young patients with BC.

Interesting, almost 25% of the patients included in the studies discussed above<sup>52,53</sup> presented increased constitutive methylation of the *BRCA1* promoter and showed similar phenotypes of germinal mutations in *BRCA1*. Hence, *BRCA1* promoter methylation may be an interesting biomarker given its clear clinicopathological correlation, and the fact that it can be detected in circulation.

#### SOMATIC MUTATION PROFILE IN YWBC

Somatic mutations have been found in all types of human cancer. In BC, the most frequently mutated genes are PIK3CA (25%), TP53 (23%), CDH1 (11%), GATA3 (7%), and PTEN (5%)54,55. In YWBC, Encinas et al.<sup>56</sup> performed a systematic review to analyze whether somatic mutations in five genes were associated with an early age at presentation of BC. They found a higher frequency of wild-type PIK3CA associated with early-onset BC, although not statistically significant when employing a multivariate model. Moreover, TP53 was mutated in 20% of tumors from both younger and older patients<sup>56</sup>. Azim et al.<sup>29</sup> examined the genomic aberrations of tumors from three different age groups, ≤45 (125 patients), 46-69 (486 patients), and ≥70 (169 patients) years of age. There was a strong positive correlation between age and somatic mutations and copy number variations (CNVs), particularly in ductal tumors. Although eleven mutations were independently associated with age at diagnosis, only mutations in GATA3 were related to young age and were twice as frequent in young patients (Fig. 2). Only one CNV event was linked to early-onset BC, with deletions in locus 6q27<sup>29</sup>.

At present, *GATA3* mutations are the main characteristic somatic aberrations detected in YWBC. GATA3 directly upregulates proto-oncogenes and ERα suggesting that it may promote tumorigenesis in luminal subtypes of cancer<sup>57</sup>. Mutations in *GATA3* affect ER binding to DNA<sup>58</sup> and modulate the response of tumor cells to estrogen signaling, which might be associated with endocrine resistance and tumor growth<sup>59,60</sup>. These results may take clinical relevance since the adverse prognosis associated with younger age at diagnosis has been observed mainly in patients with ER+ BC<sup>16</sup>.

### GERMLINE GENOMIC PROFILE IN YWBC

### **BC**-predisposing gene mutations

In cancer, early age of presentation suggests a high genetic susceptibility to the disease. In women with BC, about 1 in 10 cases represents a form of hereditary BC (Fig. 3)<sup>61,62</sup>; however, in young patients, this genetic susceptibility may be higher. Predisposing gene mutations can be classified according to the relative risk (RR) of developing cancer when a patient carries a particular germline mutation. Highly penetrant mutations are associated with a cancer RR > 5.0, moderate penetrant mutations RR ranges from 1.5 to 5.0, and low-penetrant loci changes are associated with an estimated RR of 1.5 (usually polymorphisms)<sup>63</sup>.

### Highly penetrant genes

Mutations in highly penetrant genes are responsible for the most common autosomal dominant hereditary cancer syndromes; these genes are involved in critical steps of DNA repair, apoptosis and cell proliferation (Table 1 and Fig. 4).





Risk Loci	High-penetrance	Moderate-penetrance	Low-penetrance
Gens	BRCA1/2, PT53, PTEN, STK11, CDH1	CHEK2, PALB2, ATM, BRIP1, BARD1, MRN complex, RAD51 and paralogs	10q26.13 (FGFR2), 2q33 (CASP8), 5q11.2; (MAP3K1), 11P15.5 (LSP1), 16q12.1 (TNRC9), 6q25 (ESR1), 14q24 (RAD51L1), 2q35, 8q24, 5p12, 1p11
Cancer risk (RR)	>5	1.5-5	1.1-1.5
Functional effect Population frequency	Direct effect of mutation < 0.1%	Direct effect of candidate gene < 1%	Linkage disequilibrium with causal variants >10%
Strategy for identification	Linkage and positional cloning; resequencing of candidate genes	Resequencing of candidate genes	Case-control studies; genome-wide association study

Table 1. Genetic mutations in YWBC.

YWBC: young women with breast cancer; ATM: ataxia Telangiectasia Mutated; BARD1: BRCA1 associated RING domain 1; FGFR2: fibroblast growth factor receptor 2

Figure 3. Genetic susceptibility to breast cancer.



Figure 4. Relative frequency of mutations in cancer associated genes detected by next generation sequencing in young women with breast cancer.



#### BRCA1/2

Mutations leading to the premature termination of the BRCA1/2 proteins are responsible for the Hereditary Breast and Ovarian Cancer syndrome. The lifetime risks of BC can be as high as 80% in women carrying a BRCA1 mutation<sup>64</sup>. The chance of carrying a germline mutation in BRCA1/2 in very YWBC is almost 10% for patients without a family history of BC and 12% for patients with a family history of BC65. In young patients with negative ER subtype BC and high-grade tumors, the probability of having a BRCA1 mutation is close to 30%66,67. Significant research has been made to describe the recurrent mutations in Mexican women with BC, and more than 100 deleterious variants have been found to occur among our population<sup>66</sup>. In unselected women with BC, Torres-Mejía et al. described 20 mutations in BRCA1 and 15 in BRCA2. Of these mutations, 63% were recurrent, including the deletion of exons 9-12

in *BRCA1* (ex9-12del), considered a founder mutation in the Mexican population, which accounted for almost 25% of all the mutations in *BRCA1*<sup>68</sup>. Villarreal-Garza et al. described *BRCA1/2* mutations among Mexican YWBC with triple-negative BC. 23% of the 190 patients tested carried a *BRCA* mutation; 43 mutations accounted for 89% of the total mutations, and *BRCA1* ex9-12del accounted for 41% of all the mutations detected<sup>66</sup>.

## TP53

Mutations in *TP53* gene are highly penetrant and associated with a variety of human cancers in the spectrum of the Li-Fraumeni syndrome<sup>67</sup>. The lifetime risk of BC for carriers of mutations in *TP53* is up to 50%<sup>69</sup>. The reported frequency of TP53 mutations in women diagnosed before 35 years of age ranges from < 1% to  $7\%^{69-73}$  and up to 30% for patients diagnosed before the age of 30 years.

### PTEN

Germline mutations in *PTEN* lead to the clinical manifestations collectively labeled as PTEN hamartoma tumor syndrome<sup>74</sup>. Women with mutations in *PTEN* have a BC risk in the range of 70-85%<sup>75,76</sup>, and in YWBC, the reported frequency of mutations in this gene represents <  $1\%^{77}$ .

# STK11

The cumulative incidence of BC in patients with mutations in *STK11* is approximately  $45\%^{78,79}$ . Tumors usually appear at a mean age of 41.5 years, with a higher risk in females than in males (22-fold increase) and a 20% risk of any cancer by the age of 40 compared to the general population<sup>80-82</sup>. In women with germline *STK11* gene mutations, the risk of BC by age 40 is 31%<sup>83</sup>.

# CDH1

Germline mutations in *CDH1* have been associated with hereditary diffuse gastric carcinoma<sup>84</sup>. Carriers of *CDH1* mutations face a 40-54% lifetime risk of developing BC<sup>85</sup>. Without a family history of hereditary diffuse gastric carcinoma, early-onset, and frequently bilateral BC, seems to be the highest risk factors for a mutation carrier<sup>86-89</sup>.

### Moderate-penetrance genes

Moderate-penetrance genes code for proteins that participate in complexes of DNA repair, cell cycle, and apoptosis, recruiting or interacting with polypeptides coded by highly penetrant genes. These genes include *CHEK2*, *PALB2*, *ATM*, and BRCA1 interacting protein c-terminal helicase 1 (*BRIP1*), among others (Table 1 and Fig. 4).

## CHEK2

Mutations in *CHEK2* increase BC risk from 3- to 5-fold<sup>90,91</sup>. The most studied germline mutation, c.1100delC, significantly augments the risk of early-onset and familial BC<sup>91</sup>. Other mutations have been reported in very young Pakistani women (p.P92R, p.R406C, p.H371Y, and p.D438Y)<sup>92,93</sup>, and in Chinese very young women (1169A > G)<sup>94</sup>.

### PALB2

Mutations in the *PALB2* increase the risk of BC in 9- to 10-fold in women younger than 40 compared to the general population<sup>95</sup>. According to different ethnic reports<sup>95-100</sup>, about 1% of women with early-onset BCs negative for *BRCA1/2* mutations carry a mutation in this gene.

# ATM

Women younger than 50 years who are heterozygote carriers of deleterious variants of the *ATM* gene have a 5-fold higher risk for BC<sup>101</sup>. Mutations in the *ATM* gene have been described in YWBC and are considerably more frequent when there is a familial history<sup>102-107</sup>.

### BRIP1

Mutations in the *BRIP1* gene seem to be more frequent in women with early-onset BC and triple-negative BC<sup>108</sup>. About 1% of patients with early-onset or familial BC carry a deleterious mutation<sup>103</sup>.

# BRCA1 associated RING domain 1 (BARD1)

The most described mutation in *BARD1* gene is Cys557Ser; this variant is commonly found in women younger than 50 years from Nordic population<sup>109,110</sup>. In Finnish families with breast and ovarian cancer, the variant 557Ser has a frequency of 7.4%<sup>109</sup>. In an

Icelandic population, the mutant allele 557Ser had a 3.7% frequency in cases with a family history of BC, early-onset BC, or multiple primary BCs<sup>110</sup>.

#### **MRN** complex

The MRN complex is composed of dimers of the three proteins encoded by the *MRE11A*, *RAD50*, and *NBN* genes<sup>111</sup>. Deleterious mutations have been identified in all three genes of the MRN complex. The study done by Damiola et al., which included 1313 women < 45 years old with BC, found that rare MRN gene variants significantly contribute to early-onset BC susceptibility<sup>112</sup>. NBN has the strongest evidence of acting as a BC risk gene<sup>113,114</sup>. The risk of developing any malignancy by the age of 20 in patients with NBS is > 40%<sup>115</sup>.

## RAD51 and paralogs

The RAD51 family comprises five paralogous proteins, RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3, which transduce the DNA damage signal to promote break repair<sup>116</sup> and interacts with p53<sup>117</sup>, BRCA1<sup>118</sup>, BRCA2<sup>119</sup> and PALB2<sup>120</sup> pathways. The results of a study in French patients with early-onset or familial breast and/or ovarian cancers negative for *BRCA1/2* mutations found two probable deleterious missense variants: RAD51B c.452+3A > G and RAD51C c.706-2A > G (< 45 years old), and three splicing mutations: RAD51C c.1026+5\_1026+7del, RAD51B c.475C > T/p.Arg159Cys (< 50 years old), and XRCC3 c.448C > T/p.Arg150Cys<sup>121</sup>.

### Low-penetrance BC LOCI

The polygenic model has emerged as an efficient tool to allow the detection and assessment of small risk loci when highly- and moderately-penetrant mutations cannot explain the phenotype (Table 1)<sup>122-125</sup>. Low-penetrance loci are more commonly found in genome-wide association studies (GWAS).

### GWAS

GWAS analyze considerable amounts of genomic data on large population groups; most of GWAS conducted in BC patients are focused in postmenopausal women of Caucasian ancestry, making it difficult to assess an overall risk for a particular genetic variant, and leaving other age and ethnic groups underrepresented<sup>122,125</sup>. There are two GWAS that the present data of early-onset BC<sup>122,123</sup>. The study by Ahsan et al., which included 3523 early-onset BC cases, detected 12 independently associated single nucleotide polymorphisms (SNPs); 11 of these SNPs were in the 5q11.2 locus, within or near the *MAP3K1* gene. The other locus was the phosphofructokinase muscle (*PFKM*) gene on chromosome 12q13.11. This study also found 32 additional risk loci shared between early- and late-onset BC<sup>123</sup>. The second GWAS, which included Caucasian and African-American women with BC, detected a significant association between the *GTGT* haplotype (rs11200014, rs2981579, rs1219648 and rs2420946) in fibroblast growth factor receptor 2 and the risk of early onset BC<sup>124</sup>.

# Predictive and prognostic genetic variants

The expression of the BRCA1/BRCA2/Rad51 complex is essential in the homologous recombination repair pathways; this complex has proved to be a useful prognostic biomarker in early onset BC. In the study conducted by Söderlund et al., the patients with low expression of the BRCA1/BRCA2/Rad51 complex had more local recurrences, high histologic grade, and good response to radiotherapy compared to patients with high expression of the complex<sup>126</sup>. The polymorphism 135G > C in the RAD51 gene showed functional effects and was associated with the expression of the complex. The homozygous (GG) patients had a better response to radiotherapy with a decreased risk of local recurrence. In carriers of the C allele, cyclophosphamide-methotrexate-5-fluorouracil (CMF) chemotherapy reduced the risk of distant recurrence. These results suggest that the RAD51 135G > C polymorphism could be a predictive biomarker for effective CMF chemotherapy in early-onset BC<sup>127</sup>.

Tumor necrosis factor alpha (TNF $\alpha$ ) is a pleiotropic cytokine which can regulate a wide variety of cellular responses; low concentrations of TNF $\alpha$  seem to increase tumor growth and progression<sup>128</sup>. The *TNFA*-308G > A polymorphism has been found associated with BC survival. Women heterozygous (GA) for this SNP showed a significant disadvantage in progression-free, metastasis-free, and OS compared to homozygous (GG) women<sup>129</sup>. In Mexican patients with BC, the frequency of the AA genotype is nearly 14-times higher than in the general population<sup>130</sup>.

#### PERSPECTIVES AND CHALLENGES

As discussed in this review, there is evidence showing that breast tumors in young women are more aggressive and more lethal, and that they present unique biological and molecular features at somatic and germline levels. Breast tissue is subject to continuous changes driven by hormones during the reproductive years and, therefore, pregnancy and breastfeeding may impact not only on BC risk but also on unique BC phenotypes and tumor cell biology. However, in most cases, clinical management remains the same regardless of the age at diagnosis. Thus, there is a need to develop a science-driven approach to refine and personalize treatment for YWBC.

miRNAs and epigenetic modifications arise as potentially relevant biomarkers for risk, early diagnosis, and prognosis that can be found in liquid biopsies. The research of novel agents to regulate miRNA expression will also allow targeting miRNAs as therapeutic molecules for the treatment of BC<sup>37</sup>.

Given that constitutional mutations in genes associated with hereditary BC are more common in YWBC than in postmenopausal patients with BC, international guidelines have recommended that every woman with BC under the age of 40 should be offered genetic counseling before initiating treatment<sup>131</sup>. Although most hereditary BC is attributed to mutations in *BRCA1* and *BRCA2*<sup>132</sup>, less common hereditary BC mutations should also be considered in young women. Commercially available gene panel tests allow the detection of mutations in a variety of genes associated with BC; these genomic data empower the clinician and the patient for making informed decisions about treatment and follow-up as well as cancer prevention in other family members<sup>133</sup>.

YWBC is a growing burden in Latin America, including Mexico, and research studies indicate that unique molecular biological features of this cancer are associated with age. An optimal characterization of somatic and constitutional genomic signatures offers the potential to identify targetable driver mutations. This effort will improve the treatment and prognosis of YWBC.

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